IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of :
SEKIGUCHI ET AL :
Serial No. 10/812,075:
Examiner: Cecilia M. Jaisle : Art Unit 1624
For: NOVEL QUINOLINE, TETRAHYDROQUINAZOLINE, AND PYRIMIDINE DERIVATIVES AND METHODS OF TREATMENT RELATED TO THE USE THEREOF

DECLARATION

Assistant Commissioner of Patents, Washington D.C.
Sir:
I, Kosuke Kanuma hereby declare the following:

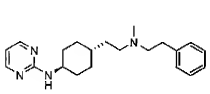
1. I am a citizen of Japan residing at 1436-1-D101 Nishiowa, Washimiya-machi, Kitakatsushika-gun, Saitama 340-0206, Japan. I graduated from Tokyo University of Science, Tokyo, Japan in March 1994. Since April 1994, I have engaged in Medical Research Laboratories, Taisho Pharmaceutical Co., Ltd.

2. I am a member of the co-inventors of the instant application, and know well the substance of the said patent application.

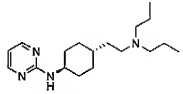
3. Under my direction and control, the following studies were performed:
Experimental Example:
Method

HEK293 cells stably expressing constitutively activated human MCH1 receptor were seeded $3x10^4$ cells/100 μL of complete culture media (Dulbecco's modified Eagle medium with 10 % fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 0.5 mg/mL G418) into poly-D-lysine pretreated black/clear bottom plates 24 hrs before the assay. Prior to the assay, the media were removed and HEK293 cells were loaded with 2 μM Fluo-4/AM calcium-sensitive dye in loading buffer (Hank's balanced salt solution supplemented with 0.5 mM probenecid, 0.05 mg/mL Amaranth, 20 mM HEPES, pH 7.4, and 0.2 % bovine serum albumin) for 1 hr at 37 °C in a 5 % CO_2 incubator. The loading buffer was removed, and fresh buffer containing various concentrations of reference

compound 1 and 2 was added to the cells, and the cells were incubated for 30 min at 37 °C. Fluorescence emission caused by increases in intracellular calcium mobilization elicited by 50 nM MCH was measured with FDSS6000™ system.



Compound 1



Compound 2

Result

The reference compound 1 and 2 did not have significant antagonist activity at human MCH1 receptor at 10 μM.

	% OF CONTROL	
conc.(M)	Compound 1	Compound 2
1.E-09	108.0	107.7
1.E-08	114.2	115.5
1.E-07	110.4	111.3
1.E-06	113.2	112.8
1.E-05	110.0	112.0
IC50(M)	>1E-5	>1E-5

4. Our compounds are recognized to be more effective in terms of the IC50 value than the reference compounds. Specifically, Examples 18 and 19 in the application identified above show IC50 value of class 1 defined in the application identified above.

5. I further state that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Signed this 12th day of March, 2008

Kosuke Kanuma

Kosuke Kanuma